SUPPORT FOR THE AMENDMENT

Claim 8 has been amended to recite that "the antibody is other than the monoclonal antibody produced by the hybridoma deposited under the accession number FERM BP-5233." While that limitation is not explicitly set forth in the specification, Applicants respectfully submit that one would have appreciated that the inventors had possession of the subject matter recited in Claim 8 as amended.

The antigen protein that the monoclonal antibody (RS38) of the present invention recognizes has subsequently been called Bst-2 (see Attachment 1 submitted herewith: Ishikawa J. et al., 1995, Genomics, 26, pp. 527-534), and it has been demonstrated by Ohtomo, T., et al., 1999, Biochem. Biophys. Res. Comm., 258, pp. 583-591 (see Attachment 2 submitted herewith) that this has the same amino sequence as the HM1.24 antigen that the monoclonal antibody "HM1.24" recognizes as described in Goto T., et al., 1991, Jpn. J. Clin. Immun., 15(6), pp. 688-691 (of record), which was published prior to the priority date of the present application.

It is stated in WO 98/35698 (see Attachment 3 submitted herewith, corresponding to European Laid-open Patent EP 0 997 152 A1, page 6) that the hybridoma that produces this monoclonal antibody "HM1.24" was internationally deposited under the provisions of the Budapest Treaty as FERM BP-5233 at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology.

In view of the foregoing, Applicants added the limitation of excluding the monoclonal antibody produced by the hybridoma deposited under the accession number FERM BP-5233 from the monoclonal antibody of Claim 8. Therefore, no new matter is believed to have been added to this application by the amendment.

REMARKS

Claims 8-10 are pending in this application.

The rejection of Claims 8 and 9 under 35 U.S.C. §102(a) over Goto et al. in light of WO 98/35698 is believed to be obviated by the amendment submitted above.

First, in regard to the antigen protein that the monoclonal antibody of the present invention recognizes, Goto et al. states that "HM1.24" recognizes is "a membrance glycoprotein with a molecular weight of 30 kD." The amino acid sequence has not been specified; it cannot be called an isolated protein; and it appears that multiple proteins "with a molecular weight of 30 kD" exist. Therefore, because the protein described in Goto et al. cannot be determined from that reference to be the same as a polypeptide which comprises the amino acid sequence of sequence SED ID NO. 1 as claimed, the antigen protein has novelty.

Further, the Examiner indicates that the monoclonal antibody HM1.24 disclosed against a specific protein in Goto et al. anticipates the instant claims, because the HM1.24 protein is 100% identical to the instant protein of SEQ ID NO.:1 as disclosed by WO 98/35698. Applicants note that the publication date of WO 98/35698 is August 20, 1998, which is subsequent to the priority date of the present application and, therefore, that publication is not available as a reference against the present application. In regard to the monoclonal antibody of Claims 8 and 9, because the antigen protein therein has novelty as described above, the monoclonal antibody that recognizes that antigen protein also has novelty.

Nonetheless, Goto et al. describes the deposit No. for the hybridoma which produces the monoclonal antibody HM1.24. As a result of an analysis, Ohtomo et al. (of record), which was published after the priority date of the present application, determined that the

amino acid sequence of the antigen protein that monoclonal antibody HM1.24 recognizes is the same protein as the antigen protein Bst-2 of the present application.

Thus, as discussed above, the limitation of excluding monoclonal antibody HM1.24 has been added to Claim 8 of the present application. The monoclonal antibody HM1.24 is described in Goto et al., and, therefore, the monoclonal antibody of amended Claim 8 is not unpatentable over that reference.

Because the hybridoma producing the monoclonal antibody RS38, which was obtained in an embodiment of the present invention, is deposited separately from that producing the monoclonal antibody HM1.24 as FERM BP-4433 at the Institute as described in line 6 from the bottom of page 21 to line 6 of page 22 of the present application, the monoclonal antibody RS38 is comparable to the monoclonal antibody HM1.24 described in Goto et al., and can be clearly recognized as a different monoclonal antibody.

Based on the foregoing, withdrawal of this ground of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

James J. Kelly, Ph.D.
Registration No. 41,504

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413 -2220
(OSMMN 08/03)

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

(2) Attachment 1

添付(1)

GENOMICS 26, 527–534 (1995)

Molecular Cloning and Chromosomal Mapping of a Bone Marrow Stromal Cell Surface Gene, BST2, That May Be Involved in Pre-B-Cell Growth

JUN ISHIKAWA,*[,1] TSUNEYASU KAISHO,*[,1] HITOSHI TOMIZAWA,* BYUNG OK LEE,* YOSHIKO KOBUNE,* JOHJI INAZAWA,† KENJI ORITANI,* MOTOYUKI ITOH,* TAKAHIRO OCHI,‡ KATSUHIKO ISHIHARA,* AND TOSHIO HIRANO*[,2]

*Division of Molecular Oncology, Biomedical Research Center and ‡Environmental Medicine, Osaka University Medical School, Suita City, Osaka 565, Japan; and †Department of Hygiene, Hematology and Oncology Unit, Kyoto Prefectural University of Medicine, Kyoto City, Kyoto 602, Japan

Received September 19, 1994; accepted January 16, 1995

Bone marrow stromal cells regulate B-cell growth and development through their surface molecules and cytokines. In this study, we generated a mAb, RS38, that recognized a novel human membrane protein, BST-2, expressed on bone marrow stromal cell lines and synovial cell lines. We cloned a cDNA encoding BST-2 from a rheumatoid arthritis-derived synovial cell line. BST-2 is a 30- to 36-kDa type II transmembrane protein, consisting of 180 amino acids. The BST-2 gene (HGMW-approved symbol BST2) is located on chromosome 19p13.2. BST-2 is expressed not only on certain bone marrow stromal cell lines but also on various normal tissues, although its expression pattern is different from that of another bone marrow stromal cell surface molecule, BST-1. BST-2 surface expression on fibroblast cell lines facilitated the stromal cell-dependent growth of a murine bone marrow-derived pre-B-cell line, DW34. The results suggest that BST-2 may be involved in pre-B-cell growth. © 1995 Academic Press, Inc.

INTRODUCTION

B lymphopoiesis occurs from hematopoietic stem cells in the bone marrow (BM) (reviewed in Whitlock *et al.*, 1985; Kincade *et al.*, 1989). This process is characterized by successive rearrangement of the immunoglobulin (Ig) loci, expression of surface Ig, and the change of growth requirement. The growth of pre-pro-B cells is dependent on contact with stromal cells but not on IL-7. During the transition from pro-B to pre-B cells, the growth dependency on stromal cells decreases and that on IL-7 increases. Subsequently, pre-B cells differentiate to B cells whose growth is independent of IL-7 (Hayashi *et al.*, 1990; Hardy *et al.*, 1991). BM stromal cells play critical roles in B lymphopoiesis by producing a variety of cytokines such as IL-6 (Hirayama *et al.*, 1992; Gimble *et al.*, 1989), IL-7 (Namen *et al.*, 1988; McNiece *et al.*, 1991; Funk *et al.*, 1993; Sudo *et al.*, 1993) and stem cell factor (Witte, 1990; Flanagan *et al.*, 1991; Martin *et al.*, 1990; Rolink *et al.*, 1991). In addition to these, there are several positive or negative regulators of B-cell formation: positive regulators include pre-B-cell growth stimulating factor (PBSF)/stromal cell-derived factor-1α (SDF-1α), pre-B-cell colony-enhancing factor (PBEF), insulin-like growth factor-I (IGF-I), and bone marrow stromal cell antigen 1 (BST-1), while sex hormones can serve as negative regulators (Nagasawa *et al.*, 1994; Tashiro *et al.*, 1993; Samal *et al.*, 1994; Landreth *et al.*, 1992; Kaisho *et al.*, 1994; Medina *et al.*, 1993; Kincade, 1994). Direct interactions between B lineage cells and BM stromal cells also are critical for early B-cell development (Dorshkind, 1990; Kierney and Dorshkind, 1987). For example, anti-CD44 mAb inhibits lymphoid cell generation in murine long-term BM cultures (Miyake *et al.*, 1990). Furthermore, VCAM-1 is involved in the *in vitro* binding between murine BM stromal cells and B precursor cell lines (Miyake *et al.*, 1991). However, as-yet unidentified stromal cell-derived molecules are involved in B lineage cell growth and development in both murine (Cumano *et al.*, 1990; Gunji *et al.*, 1991; Palacios and Samaridis, 1992) and human (Wolf *et al.*, 1991; Kaisho *et al.*, 1992; Moreau *et al.*, 1993) culture systems.

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic polyarthritis, synovial cell proliferation, hypergammaglobulinemia, and polyclonal B-cell activation. Several cytokines that stimulate lymphocyte activation are overproduced in the synovium and may be related to the pathogenesis of RA

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. D28137.

[1] The first two authors contributed equally to this work.

[2] To whom correspondence should be addressed at the Division of Molecular Oncology, Biomedical Research Center, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan. Telephone: 81-6-879 3880. Fax: 81-6-879-3889.

0888-7543/95 $6.00
Copyright © 1995 by Academic Press, Inc.
All rights of reproduction in any form reserved.

(3) Attachment 2

資料(2)

Biochemical and Biophysical Research Communications 258, 583–591 (1999)
Article ID bbrc.1999.0683, available online at http://www.idealibrary.com on IDEAL®

Molecular Cloning and Characterization of a Surface Antigen Preferentially Overexpressed on Multiple Myeloma Cells

Toshihiko Ohtomo,* Yasuhiro Sugamata,* Yasuko Ozaki,* Koichiro Ono,* Yasushi Yoshimura,* Shigeto Kawai,* Yasuo Koishihara,* Shuji Ozaki,† Masaaki Kosaka,† Toshio Hirano,‡ and Masayuki Tsuchiya*[1]

*Chugai Pharmaceutical Co., Ltd., Fuji-Gotemba Research Labs., 1-135 Komakado, Gotemba-shi, Shizuoka 412-8513, Japan; †The First Department of Internal Medicine, School of Medicine, University of Tokushima, 3-8-15 Kuramoto-cho, Tokushima 770-8503, Japan; and ‡Department of Oncology, Biomedical Research Center, Osaka University Medical School, Suita, Osaka 565-0871, Japan

Received April 14, 1999

HM1.24 antigen has been identified as a surface molecule preferentially expressed on terminally differentiated B cells, and its overexpression is observed in multiple myeloma cells. The HM1.24 antigen is, therefore, expected as a most potent target molecule for antibody-based immunotherapy for multiple myeloma. Here, we have identified the cDNA for human HM1.24 antigen and also analyzed its gene structure including the promoter region. The HM1.24 antigen is a type II membrane glycoprotein, which has been reported as a bone marrow stromal cell surface antigen BST2, and may exist as a homodimer on myeloma cell surface. Although a reason for the overexpression in myeloma cells is not understood, very interestingly, the promoter region of the HM1.24 gene has a tandem repeat of three cis elements for a transcription factor, STAT3, which mediates interleukin-6 (IL-6) response gene expression. Since IL-6 is a differentiation factor for B cells, and known as a paracrine/autocrine growth factor for multiple myeloma cells, the expression of HM1.24 antigen may be regulated by the activation of STAT3. Importantly, a humanized anti-HM1.24 antibody effectively lysed the CHO transformants which expressed HM1.24 antigen as high as human multiple myeloma cells, but not the cells with lower antigen expression. This evaluation shows that ADCC heavily depends on the expression level of target antigens and, therefore, the immunotherapy targeting the HM1.24 antigen should have a promising potential in clinical use. © 1999 Academic Press

Multiple myeloma (MM) is a lethal disease characterized by a clonal accumulation of plasma cells and usually accompanied by homogeneous immunoglobulin in the serum and/or urine. Bone marrow invasion by these tumor cells is associated with severe anemia and humoral immunodeficiency resulting in concomitant bacterial infections (1). In addition, an abnormal cytokine environment such as elevated IL-6 and/or IL-1β as well as TNFα, often results in increased osteolysis leading to bone pain, pathologic fractures, and hypercalcemia (2). Since a variety of chemotherapies have had little impact on the overall clinical course, alternative approaches against multiple myeloma are greatly needed (3). Indeed, several mouse monoclonal antibodies have been established to target cell surface antigens such as CD38 (4, 5) and CD54 (6) on myeloma cells, or been shown to inhibit the factor-dependent cell growth of MM cells (7). Furthermore, humanized anti-CD38 (8), anti-interleukin-6 (IL-6) and anti-IL-6 receptor antibodies (9–11) have shown their potential in therapeutic use against MM.

However, CD38 and CD54 are also found on a variety of cells including hematopoietic stem cells (12, 13), leading to serious reservations that these molecules can not be suitable target antigens for immunotherapy of plasma cell dyscrasias in terms of adverse effects. Furthermore, although IL-6 has been reported to be a major growth factor of MM cells, some myeloma cells do not proliferate in response to IL-6, and the inability of anti-IL-6 antibody to block proliferation of certain MM cells is known (14, 15).

To develop a novel immunotherapeutic strategy, we have generated a mouse monoclonal antibody, which is highly specific for a surface antigen of multiple my-

[1] To whom requests for reprints should be addressed. Fax: (81)-550-87-5397. E-mail: tsuchiyamsy@gt.chugai-pharm.co.jp.

0006-291X/99 $30.00
Copyright © 1999 by Academic Press
All rights of reproduction in any form reserved.

Received at: 5:30AM, 11/25/2003

2003年11月25日 19時14分 SIP&WAY NO. 2128 P. 11 資料(3)

(4) Attachment 3

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 997 152 A1

(12) EUROPEAN PATENT APPLICATION
published in accordance with Art. 158(3) EPC

(43) Date of publication:
03.05.2000 Bulletin 2000/18

(21) Application number: 98802757.8

(22) Date of filing: 12.02.1998

(51) Int. Cl.[7]: A61K 39/395, C07K 16/30, C07K 16/46 // C12N15/13, C12P21/08 ,(C12P21/08, C12R1:91)

(86) International application number:
PCT/JP98/00568

(87) International publication number:
WO 98/35698 (20.08.1998 Gazette 1998/33)

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 12.02.1997 JP 4141097

(71) Applicant: CHUGAI SEIYAKU KABUSHIKI KAISHA
Tokyo, 115-8543 (JP)

(72) Inventors:
- KOISHIHARA, Yasuo, Chugai Seiyaku K.K.
Gotenba-shi Shizuoka 412-8513 (JP)
- YOSHIMURA, Yasushi, Chugai Seiyaku K.K.
Gotenba-shi Shizuoka 412-8513 (JP)

(74) Representative:
Wakerley, Helen Rachael et al
Reddie & Grose,
16 Theobalds Road
London WC1X 8PL (GB)

(54) REMEDIES FOR LYMPHOCYTIC TUMORS

(57) A therapeutic agent for lymphatic tumors (excluding myeloma) comprising as an active ingredient an antibody that specifically binds to a protein having the amino acid sequence as set forth in SEQ ID NO: 1 and that has a cytotoxic activity.

EP 0 997 152 A1

having the activity of binding to HM1.24 antigen or HM1.24 antigen-expressing cells (see Japanese Post-examined Patent Publication (Kokoku) No. 1-59878). Furthermore, a transgenic animal having a repertoire of all human antibody genes is immunized with the antigen, i.e., HM1.24 antigen or HM1.24 antigen-expressing cells, to obtain the desired humanized antibody in the method described above (see International Patent Applications WO 93/12227, WO 92/03918, WO 94/02602, WO 94/25585, WO 96/34096 and WO 96/33735).

The monoclonal antibody-producing hybridomas thus constructed can be subcultured in a conventional culture medium, or can be stored for a prolonged period of time in liquid nitrogen.

In order to obtain the monoclonal antibody from said hybridoma, there can be mentioned a method in which said hybridoma is cultured in a conventional method and the antibodies are obtained in supernatant, or a method in which the hybridoma is administered to and grown in a mammal compatible with said hybridoma and the antibodies are obtained in the ascites. The former method is suitable for obtaining high-purity antibodies, whereas the latter is suitable for a large scale production of antibodies.

Specifically the anti-HM1.24 antibody-producing hybridoma can be constructed using: the method of Goto, T. et al. (Blood (1994) 84: 1922-1930). It can be conducted by a method in which the anti-HM1.24 antibody-producing hybridoma that was internationally deposited under the provisions of the Budapest Treaty as FERM BP-5233 on September 14, 1995 with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, of 1-3, Higashi 1-chome, Tsukuba city, Ibaraki pref., Japan, is intraperitoneally injected to BALB/c mice (manufactured by CLEA Japan) to obtain the ascites from which the anti-HM1.24 antibody is purified, or: a method in which said hybridoma is cultured in a suitable culture medium such as the RPMI1640 medium containing 10% fetal bovine serum and 5% BM-Condimed H1 (manufactured by Boehringer Mannheim), the hybridoma SFM medium (manufactured by GIBCO-BRL), the PFHM-II medium (manufactured by GIBCO-BRL) and the like, and the anti-HM1.24 antibody can be purified from the supernatant.

1-2. Recombinant antibody

A recombinant antibody which was produced by the recombinant gene technology in which an antibody gene was cloned from the hybridoma and integrated into a suitable vector which was then introduced into a host can be used in the present invention as monoclonal antibody (see, for example, Carl, A.K., Borrebaeck, and James, W. Larrick, THERAPEUTIC MONOCLONAL ANTIBODIES, published in the United Kingdom by MACMILLAN PUBLISHERS LTD. 1990).

Specifically, mRNA encoding the variable region (V region) of the desired antibody is isolated from the hybridoma producing the antibody. The isolation of mRNA is conducted by preparing total RNA using, for example, a known method such as the guanidine ultracentrifuge method (Chirgwin, J.M. et al., Biochemistry (1979) 18, 5294-5299), the AGPC method (Chomczynski, P. et al., Analytical Biochemistry (1987) 162, 156-159), and then mRNA is purified from the total RNA using the mRNA Purification kit (manufactured by Pharmacia) and the like. Alternatively, mRNA can be directly prepared using the Quick Prep mRNA Purification Kit (manufactured by Pharmacia).

cDNA of the V region of antibody may be synthesized from the mRNA thus obtained using a reverse transcriptase. cDNA may be synthesized using the AMV Reverse Transcriptase First-strand cDNA Synthesis Kit and the like. Alternatively, for the synthesis and amplification of cDNA, the 5'-Ampli FINDER RACE Kit (manufactured by Clontech) and the 5'-RACE method (Frohman, M.A. et al., Proc. Natl. Acad. Sci. U.S.A. (1988) 85, 8998-9002; Belyavsky, A. et al., Nucleic Acids Res. (1989) 17, 2919-2932) that employs polymerase chain reaction (PCR) may be used. The desired DNA fragment is purified from the PCR product obtained and may be ligated to vector DNA. Moreover, a recombinant vector is constructed therefrom and then is introduced into E. coli etc., from which colonies are selected to prepare a desired recombinant vector. The nucleotide sequence of the desired DNA may be confirmed by a known method such as the dideoxy method.

Once the DNA encoding the V region of the desired antibody has been obtained, it may be ligated to DNA encoding the constant region (C region) of the desired antibody, which is then integrated into an expression vector. Alternatively, the DNA encoding the V region of the antibody may be integrated into an expression vector which already contains DNA encoding the C region of the antibody.

In order to produce the antibody for use in the present invention, the antibody gene is integrated as described below into an expression vector so as to be expressed under the control of the expression regulatory region, for example an enhancer and/or a promoter. Subsequently, the expression vector may be transformed into a host cell and the antibody can then be expressed therein.

1-3. Altered antibody

In accordance with the present invention, artificially altered recombinant antibody such as chimeric antibody and humanized antibody can be used for the purpose of lowering heterologous antigenicity against humans. These altered antibodies can be produced using known methods.

Chimeric antibody can be obtained by ligating the thus obtained DNA encoding a V region of antibody to DNA encoding a C region of human antibody, which is then inserted into an expression vector and introduced into a host for production of